NOVEL SMALL ACTIVATING RNA

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a Section 371 of International Application No. CT/CN2019/082149, published in the Chinese language on Oct. 17, 2019, under International Publication No. WO 2019/196887 A1, which claims priority to Chinese Application No. 201810317366.X, filed on Apr. 10, 2018, the disclosure of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "065786_1US1_Sequence_Listing_Sustitute" and a creation date of Oct. 28, 2020 and having a size of 115 KB. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention relates to the field of molecular biology, and in particular to up-regulating gene expression with a double-stranded small RNA targeting a gene promoter.

BACKGROUND OF THE INVENTION

[0004] Double-stranded small nucleic acid molecules including chemically synthesized oligoribonucleotides (such as small activating RNA (saRNA)) and naturally occurring oligoribonucleotides (such as micro ribonucleic acid (miRNA)) have been proved to be capable of targeting regulatory sequences (such as promoter sequences) of protein-coding genes in a sequence-specific manner to up-regulate gene expression at the transcriptional and epigenetic level, a phenomenon known as RNA activation (RNAa) (Li, Okino et al. (2006) Proc Natl Acad Sci USA 103:17337-17342; Janowski, Younger et al. (2007) Nat Chem Biol 3:166-173; Place, Li et al. (2008) Proc Natl Acad Sci USA 105:1608-1613; Huang, Place et al. (2012) Nucleic Acids Res 40:1695-1707; Li (2017) Adv Exp Med Biol 983:1-20). Studies have shown that RNAa is an endogenous molecular mechanism evolutionarily conserved from *Caenorhabditis elegans* to the human being (Huang, Qin et al. (2010) PLoS One 5:e8848; Seth, Shirayama et al. (2013) Dev Cell 27:656-663; Turner, Jiao et al. (2014) Cell Cycle 13:772-781).

[0005] Safe strategies to selectively enhance gene and/or protein production remain a challenge in gene therapy. Viral-based systems have inherent drawbacks including adverse effects on host genome integrity and immunological consequences. RNAa, with the advantages of being able to activate endogenous genes without the risk of altering the genome, represents a new strategy to stimulate target gene expression.

[0006] Cyclin-dependent kinase (CDK) inhibitor p21WAF1/CIP1 (p21) is a mediator of several anti-growth pathways and considered a potent tumor suppressor gene in cancer cells (Harper, Adami et al. (1993) Cell 75:805-816). In fact, overexpression of p21 by ectopic vectors or stimulation of endogenous transcription inhibits tumor growth both in vitro and in vivo (Harper, Adami et al. (1993) Cell 75:805-816; Eastham, Hall et al. (1995) Cancer Res 55:5151-5155; Wu, Bellas et al. (1998) J Exp Med 187:1671-1679; Harrington, Spitzweg et al. (2001) J Urol 166:1220-1233). As such, selective activation of p21 can possess therapeutic application for regulating cell growth and treatment of disease (e.g. cancer).

SUMMARY OF THE INVENTION

[0007] The present invention provides a type of oligonucleotide molecules and a method that can upregulate gene expression in a cell in a targeted manner. By introducing into cells, the oligonucleotide molecule targeting the promoter sequence of a gene, the method can effectively upregulate the expression of the targeted gene and produce an effective biological effect. The oligonucleotides of the present invention are structurally-optimized double-stranded ribonucleic acid molecules, which is also known as small activating RNA (saRNA).

[0008] The present invention provides a saRNA, comprising a first oligonucleotide strand of 17 to 30 nucleotides in length and a second oligonucleotide strand of 17 to 30 nucleotides in length, and a region of at least 15 nucleotides in length in the first oligonucleotide strand that is complementary to the second oligonucleotide strand. The first oligonucleotide strand or the second oligonucleotide strand has more than 75% (such as more than 80%, more than 85%, more than 90%, more than 95%, more than 99%, or 100%) sequence homology or complementarity with any continuous fragment of 15 to 30 nucleotides in length in the promoter of a target gene. One end of a duplex formed by the first oligonucleotide strand and the second oligonucleotide strand is a blunt end, i.e. having no overhang structure, and the other end of the duplex can have an overhang consisting of 1 to 4 nucleotides (such as 1, 2, 3 or 4 nucleotides) formed at the terminus of the first oligonucleotide strand or the second oligonucleotide strand. In a specific embodiment, one end of the duplex formed by the first oligonucleotide strand and the second oligonucleotide strand in the aforementioned saRNA is a blunt end, and the other end can have an overhang of 2 or 3 nucleotides formed at the terminus of the first oligonucleotide strand or the second oligonucleotide strand.

[0009] In the aforementioned saRNA, nucleotides of the overhang can be selected from T (thymine), U (uracil), or a "natural" nucleotide, for example, the overhang is selected from dTdTdT, dTdT, UUU, UU, or 2 or 3 continuous natural nucleotides (such as A, T, G and C). The natural nucleotide overhang in the present invention means that nucleotides overhanging at the terminus of the first oligonucleotide strand or the second oligonucleotide strand are homologous to or complementary with nucleotides at the corresponding position in a target sequence.

[0010] In the aforementioned saRNA, the 5' terminus of the first oligonucleotide strand or the second oligonucleotide strand is at the blunt end of the duplex, and there are 1 to 3 nucleotides of the first to the third nucleotide from the 5' terminus in the first or second oligonucleotide strand mispaired with nucleotides at the corresponding position in the other strand. Preferably, the mispairing involves mispaired cytosines. There are at least two patterns for the cytosine mispairing described herein. In one pattern, the nucleotides on one strand are cytosine nucleotides (C), while the guanine nucleotides (G) to be paired with the cytosine nucleotides by